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Tissue Distribution and Correlation Profiles of Heavy-Metal Accumulation in the Freshwater Crayfish *Astacus leptodactylus*

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Abstract The present work details the analysis of heavy-metal and metalloid concentrations in exoskeleton, gill, hepatopancreas, and abdominal muscle tissues of 60 crayfish (*Astacus leptodactylus*) specimens collected from Lake Hirfanlı, a dam lake located in Kırşehir (Turkey) with a low metal-contamination profile. Concentrations of 11 metals (aluminum [Al], chromium [Cd], manganese [Mn], cobalt [Co], nickel [Ni], copper [Cu], molybdenum [Mo], silver [Ag], cadmium [Cd], mercury [Hg], and lead [Pb]) and a metalloid (arsenic [As]) were measured by inductively coupled plasma–mass spectrometry, and the relative frequencies of the most abundant isotopes of Cr, Cu, Ag, Cd, Hg, and Pb were evaluated. Three correlation trends were evaluated between the following: (1) different elements in the each individual tissue, (2) individual elements in different tissues, and (3) different elements in different tissues. In addition, correlation rates of growth parameters (weight, cephalothorax length, and total length) with heavy-metal and metalloid concentrations in each tissue were investigated. Our results suggest that substantial differences in metal and metalloid-accumulation levels exist between male and female specimens, with stronger correlations between the heavy-metal concentrations observed in the male cohort. It is notable that correlation trends of Co, Cu, ^{52}As, Cr, and Ni in exoskeleton of the male specimens display strong similarities. Likewise, a very strong correlation is present in Ni–Cd and Ni–Pb accumulations in abdominal muscle of the male specimens; a similar trend is present between Cd and Pb concentrations in the same tissue of female specimens. For correlation rates of different heavy metals and metalloid in different tissues, the strongest positive association observed was between ^{63}Cu in gill and As in hepatopancreas, whereas the strongest negative correlation was between accumulated Ni in abdominal muscle and As in exoskeleton. Strong correlations between metals and metalloid accumulations were observed between exoskeleton and gill. In many cases, metal and metalloid accumulation was negatively correlated with growth parameters. Preferential accumulation of Cr and Cu isotopes was observed in different tissues, suggesting that significant amounts of isotope fractionation occur during heavy-metal accumulation. Relatively low correlation rates were observed between ^{52}Cr/^{53}Cr and ^{63}Cu/^{65}Cu concentrations in several tissue types in both male and female cohorts, whereas no such trend was observed between Cd and Pb isotopes.

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Factory wastes released as byproducts of industrial development are prime causes of heavy-metal pollution. Due to their tendency to greatly accumulate in almost any organism and their ability to physically displace cofactors or other vital moieties in biological systems, heavy metals pose a considerable danger to human and animal health; thus, their monitoring and potential removal is of substantial importance (Kouba et al. 2010). Although the toxicity of heavy metals may vary greatly, with some being used in enzymes of various organisms (e.g., Cu in crustaceans), all invariably cause negative effects above a certain concentration. Those effects include various toxicoses and neoplastic diseases (Gadzala-Kopciuch et al. 2004; Alcorlo et al. 2006).

Analysis of heavy-metal incorporation and the associated dangers to marine or freshwater ecosystems by way of the food chain is highly important to understand the risks as well as the extent of heavy-metal pollution (Kouba et al. 2010; Tunca et al. 2012). The use of bioindicator species is one of the most precise and accurate methods available for the study of heavy-metal pollution and its effects. Previous studies indicate that crayfish are capable of accumulating heavy metals with rates dependent on the external concentration of the metal in question (Anderson et al. 1997; Lopez et al. 2004). Crayfish are also remarkably adaptable benthic, solitary omnivores displaying brood care, high fecundity, and short generation times, all of which increase the value of crayfish as a bioindicator species. Due to their position in the food web, crayfish may also facilitate the transfer of toxins and contaminants to higher trophic levels (Wigginton and Birge 2007).

Astacus leptodactylus is an important biomonitor species. Although previous studies have reported the trends of metal accumulation on this species, accumulation effects of each metal on the accumulation trends of other metals has still not been elucidated on this species and even in crayfish in general (Guner 2007; Naghshbandi et al. 2007; Kurun et al. 2010). The purpose of this study therefore was to understand the accumulation and correlation trends of several metals and a metalloid, with emphasis placed on the relative accumulation rates of metal isotopes.

In this report, we present analysis of nine different heavy metals (aluminum [Al], cadmium [Cd], cobalt [Co], copper [Cu as ^{65}Cu and ^{65}Cu], chromium [Cr as ^{52}Cr and ^{53}Cr], nickel [Ni], manganese (Mn), molybdenum [Mo], and lead [Pb]) and a metalloid (arsenic [As]) in four different tissues (gill, exoskeleton, hepatopancreas, abdominal muscle) of the freshwater crayfish *Astacus leptodactylus* and report (1) the accumulation rates of the aforementioned metals and metalloid, (2) their presence and correlation in the same tissue type, (3) the accumulation trends of their isotopes, and (4) the correlation of individual heavy-metal and metalloid ions across four different tissues and growth parameters. Regression analysis was used for the modeling of some metals and the metalloid ($R^2 > 0.80$). Mercury (Hg) and silver (Ag) were also tested for but are not listed here because the concentrations of those heavy metals were lower than the detection limits for all samples tested.

Materials and Methods

Crayfish specimens were collected from Lake Hirfanlı, a dam lake located in Kırşehir, Turkey (39° 16′ 20.28″ N, 33° 31′ 7.68″ E). Crayfish traps and trammel nets of varying mesh sizes were used for capture; all specimens were obtained in a single collecting operation on September 28, 2011. A total of 60 crayfish specimens, 37 male and 23 female, of varying sizes, were collected in total and transported in Igloo cooler boxes. Sediment samples were also collected in three different sampling stations.

Sample Preparation and Analysis

Sediment samples were dried at 100 °C for 24 h, pressed into pellets under high pressure, and analyzed for elemental composition in a Rigaku ZSX Primus II X-ray fluorescence spectrometer (Rigaku, Japan). Crayfish specimens collected in the field were stored at −20 °C in plastic container bags until dissection. Weights, cephalothorax lengths, and total lengths (excluding chelae) of all specimens were measured before excision of the cephalothorax exoskeleton, gills, hepatopancreas, and abdominal muscles. Samples of those four tissue types were then prepared for inductively coupled plasma–mass spectrometry (ICP-MS) measurements by digestion according to Bernhard (1976).

An X-Series II ICP-MS machine (Thermo Scientific, USA) equipped with ID100 Autodiluter (Thermo Scientific, USA) and Cetac Asx-260 autosampler (CETAC Technologies, USA) accessories, was used for ICP-MS analysis of the tissue samples. All dilutions were performed with a 2 % nitric acid matrix in ultrapure water. Standard curves were prepared by taking the QCS-27 series of elements (high-purity standards) as basis. Concentration ranges of the relevant elements in the tissue samples were taken into account in the preparation of standard curves; a correlation coefficient >0.99 was obtained for each calibration curve. To maximize the detection capacity and peak heights, the mass spectrometer was autotuned daily. Metal and metalloid concentrations in a total of 240 samples were measured; measurements of standards were performed after every 20 samples to ensure consistency. In addition, 10 ppb ^{209}bismuth was used as an internal standard.

Interferences created by polyatomic ions of IA and IIA metals were removed by collision cell technology. Three runs were performed for each sample, and the dwell time was 10 ms for all elements except Al, for which a dwell time of 50 ms was used due to this element's low atomic weight and consequent difficulty of detection. Sampling and washing times were 90 s each and determined by the length of tubing. ^{27}Al, ^{52}Cr, ^{53}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{75}As, ^{95}Mo, ^{107}Ag, ^{109}Ag, ^{111}Cd, ^{112}Cd, ^{113}Cd, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{206}Pb, ^{207}Pb, and ^{208}Pb were the heavy-metal and metalloid isotopes measured. All Ag and Hg isotopes were lower than detection limits for all tissues tested and were therefore omitted in the results section. Isotopes of Cd strongly correlated with each other in all samples and tissues tested and were consequently analyzed

together; Pb isotopes displayed a similar trend. However, isotopes of Cr and Cu had different levels of correlation and were therefore treated separately for correlation analysis. LUTS-1 was used as reference material for evaluating the recovery rates of As, Cd, Co, Cu, Cr, Ni, and Pb. Because As displayed a relatively low recovery rate, possibly due to sublimation of this element during preparation, values for this element are adjusted in the following analyses. Likewise, Pb had a recovery rate greater than expected, possibly due to the low concentration of this metal in the reference sample, and analyses concerning Pb were adjusted accordingly.

Statistical Analysis

Before correlation analysis, all results were subjected to Kolmogorov–Smirnov test to observe the normality of data distribution. The normality graph was evaluated; the data sets that fit a probability distribution were analyzed using Pearson's Chi-square test, whereas those that did not were analyzed using Spearman's rank test; and $P < 0.05$ and $P < 0.01$ were used as significance criteria, respectively. The strongest correlations amongst the heavy-metal and metalloid concentrations in each of the four tissues analyzed were determined by principal component analysis (PCA) as described by Varmuza and Filzmoser (2009). Multiple linear regression analysis was used to predict heavy metal concentrations in tissues. The analysis was applied to all heavy metals in all tissues, although only models with $R^2 > 0.80$ are presented in this article. Normality graphs were determined to observe data distribution patterns, and a stepwise method was chosen during the analysis. All statistical calculations were performed with the commercial statistics program SPSS 17.0 (IBM, USA).

Results and Discussion

We analysed three main correlation data: (1) the correlation between different elements accumulated in the same tissue type, (2) the correlation between the concentrations of the same element between different tissue types, and (3) the correlation between a single element and other elements in different tissue types. Analysis results for the three previously mentioned criteria are detailed in Tables 1–4, 5 and 6, respectively.

In addition, sediment samples were analyzed to determine their elemental composition, and biomagnification factors were determined for each element for which a sediment presence could be detected (Tables 7, 8). It is notable that the surrounding sediment had relatively high amounts of Al, perhaps accounting for the low biomagnification factor for this metal, which is amongst the lowest for the elements tested. ^{63}Cu and ^{65}Cu displayed particularly high biomagnification factors, which may reflect the selective accumulation of this metal in crayfish due to its presence in the blood pigment hemocyanin. Gills were the site displaying the highest bioaccumulation factors, possibly because gills are in constant contact with the outside environment and are therefore prime sites of sorption for many metal. The exoskeleton displayed similarly high results, which, although not as pronounced as the gill data, again reflect the external nature of this tissue. Hepatopancreas biomagnifications rates were high for some elements but not for others, likely reflecting the fact that certain heavy metals are actively transported to this organ for detoxification.

Correlation Trends of Different Heavy Metals and Metalloid in the Same Tissue Type

Correlation rates were described as "weak," "moderate," "strong," and "exceptionally strong" in increasing order. As could be expected, weight, cephalothorax length, and total length measurements in both male and female specimens strongly correlated with each other. Tables 1, 2, 3 and 4 list the correlation rates between different metals and metalloid within the same tissue (separately for male and female specimens) type for exoskeleton, gill, hepatopancreas, and abdominal muscle samples, respectively.

Co is an essential heavy metal for many organisms (Kwoczek et al. 2006). Despite the necessity of this element in tissues, its concentrations were lower than detection limits in gill and abdominal muscle samples and therefore were not considered for correlation analysis. This element was present in exoskeleton and hepatopancreas despite the its absence in gill and abdominal sample, possibly because the former two tissue types are known sites of bioaccumulation and therefore more likely to display greater concentrations of Co (Ahearn et al. 2004; Lacerda et al. 2009). In addition, low overall tissue levels of Co can be explained by the fact that Co bioaccumulation is inhibited in the presence of other heavy metals—especially Ni, Cu, Zn, and Mn—possibly due to competitive interactions between those cations (Norwood et al. 2007). Accumulated Co concentrations displayed a moderate negative correlation with all growth parameters in the exoskeletons of female specimens, whereas no such trend was observed in the hepatopancreatic samples of both sexes. Compared with the hepatopancreas results, Co amounts in exoskeleton correlated with more metals and with greater strength. In addition, male specimens had greater similarities between correlation trends of Co and other elements compared with female specimens. The correlation rates of Co with Pb and As are especially curious because male specimens displayed strong

Table 1 Correlation rates between different metals within the same tissue type for exoskeleton samples

	Length	CL	W	Al	Mn	Co	Ni	^{63}Cu	^{65}Cu	As	^{208}Pb	^{53}Cr	^{52}Cr
Male													
Length	1												
CL	0.937**	1											
Weight	0.927**	0.917**	1										
Al	0.398*	0.341*	0.344*	1									
Mn	0.250	0.168	0.135	0.377*	1								
Co	−0.125	−0.159	−0.103	0.017	0.104	1							
Ni	−0.282	−0.335*	−0.243	−0.055	0.040	0.738**	1						
^{63}Cu	−0.344*	−0.344*	−0.332*	0.073	0.049	0.687**	0.694**	1					
^{65}Cu	−0.447**	−0.462**	−0.398*	−0.035	−0.072	0.620**	0.633**	0.921**	1				
As	−0.331*	−0.345*	−0.290	−0.220	0.015	0.850**	0.798**	0.761**	0.715**	1			
^{208}Pb	−0.064	−0.008	−0.061	−0.064	−0.008	−0.816**	−0.520**	−0.447**	−0.411*	−0.656**	1		
^{53}Cr	−0.581**	−0.585**	−0.580**	−0.093	0.028	0.436**	0.413*	0.440**	0.463**	0.429**	−0.133	1	
^{52}Cr	−0.491**	−0.566**	−0.512**	−0.220	0.081	0.656**	0.768**	0.719**	0.792**	0.892**	−0.477**	0.675**	1
Female													
Length	1												
CL	0.957**	1											
Weight	0.903**	0.891**	1										
Al	0.171	0.233	0.115	1									
Mn	−0.037	−0.087	−0.165	−0.203	1								
Co	−0.514*	−0.581**	−0.466*	−0.161	0.145	1							
Ni	−0.443*	−0.511*	−0.327	0.095	0.065	0.801**	1						
^{63}Cu	−0.201	−0.192	−0.017	0.250	−0.220	−0.061	0.473*	1					
^{65}Cu	−0.357	−0.354	−0.182	0.151	−0.142	0.147	0.611**	0.966**	1				
As	−0.313	−0.345	−0.157	−0.063	−0.143	0.308	0.607**	0.758**	0.836**	1			
^{208}Pb	−0.054	−0.064	−0.178	−0.078	0.280	−0.120	−0.288	−0.508*	−0.513*	−0.720**	1		
^{53}Cr	−0.284	−0.389	−0.438*	−0.046	0.498*	0.727**	0.522*	−0.252	−0.071	−0.036	0.197	1	
^{52}Cr	−0.419*	−0.481*	−0.379	−0.093	−0.069	0.423*	0.515*	0.556**	0.691**	0.780**	−0.572**	0.254	1

Significant results (* $P < 0.05$), particularly notable correlations (** $P < 0.01$)

CL carapace length excluding chelae

Table 2 Correlation rates between different metals within the same tissue type for gill samples

	Length	CL	Weight	Al	^{52}Cr	^{53}Cr	Mn	Ni	^{63}Cu	^{65}Cu	As
Male											
Length	1										
CL	0.937**	1									
Weight	0.927**	0.917**	1								
Al	0.091	0.024	0.107	1							
^{52}Cr	−0.509**	−0.521**	−0.480**	0.308	1						
^{53}Cr	−0.406*	−0.355*	−0.422**	−0.150	0.404*	1					
Mn	−0.351*	−0.396*	−0.384*	0.327*	0.701**	0.246	1				
Ni	−0.352*	−0.422**	−0.349*	0.420**	0.797**	−0.040	0.683**	1			
^{63}Cu	−0.318	−0.357*	−0.255	−0.014	0.707**	0.092	0.501**	0.729**	1		
^{65}Cu	−0.326*	−0.332*	−0.268	0.014	0.815**	0.136	0.569**	0.786**	0.942**	1	
As	−0.347*	−0.368*	−0.320	0.223	0.710**	0.425**	0.575**	0.563**	0.539**	0.588**	1
Female											
Length	1										
CL	0.957**	1									
Weight	0.903**	0.891**	1								
Al	0.146	0.095	0.077	1							
^{52}Cr	−0.363	−0.364	−0.219	0.006	1						
^{53}Cr	0.096	0.134	0.165	−0.312	0.189	1					
Mn	0.034	−0.044	0.058	0.265	0.129	0.088	1				
Ni	−0.137	−0.218	−0.061	0.447*	0.656**	−0.075	0.476*	1			
^{63}Cu	−0.181	−0.187	−0.068	−0.025	0.674**	−0.020	0.275	0.664**	1		
^{65}Cu	−0.271	−0.297	−0.126	0.050	0.841**	0.056	0.365	0.776**	0.928**	1	
As	−0.225	−0.303	−0.096	0.230	0.800**	0.221	0.383	0.826**	0.542**	0.711**	1

Significant results (* $P < 0.05$), particularly notable correlations (** $P < 0.01$)

CL carapace length excluding chelae

correlation between the accumulations of those metals, whereas a similar result was not observed in female specimens. Exoskeletal Co accumulation correlations of male specimens were especially similar to those of Cu, Ni, ^{52}Cr, and As in the same tissue.

It is still unclear whether Ni is an essential metal for crayfish (Khan and Nugegoda 2003; Yilmaz and Yilmaz 2007). Essential or otherwise, Ni is toxic above a certain threshold and was the only tested metal to correlate with the essential metal Mn. In addition, strong correlations were observed between Ni–Mn, Ni–Pb, and Ni–Cd concentrations in abdominal muscle, especially for male specimens. As with Co, exoskeletal Ni correlation trends in male specimens were similar to those of Cu, Co, ^{52}Cr, and As in the same tissue.

Some inorganic forms of As, such as As(III) and As(V), are highly toxic (Fatrorini et al. 2006). As was observed to display a weak negative correlation with growth parameters in exoskeleton and gill samples from male specimens, whereas a moderate negative correlation was observed in hepatopancreas and no correlation was present between As and cephalothorax length, total length, or weight in abdominal muscle samples. For female specimens, it is curious that moderate and positive weak correlations were observed in As concentrations of abdominal muscle with weight and total length, respectively, although the other three tissue types did not display any similar correlation. Correlations of As with ^{52}Cr and Co in the exoskeletal samples from male specimens are notable. In addition, whereas a lower number of metals were found to correlate with As amounts in gill and muscle tissues of female specimens, these correlations were stronger compared with those found in the same tissue types of male specimens. As, Cu, Co, ^{52}Cr, and Ni again have similar correlation parameters in the exoskeletal samples of male specimens; for As, this correlation trend is also observed alongside Cu and Cr in exoskeleton samples from female specimens.

Pb, a nonessential and carcinogenic metal (Alcorlo et al. 2006), displays unusual results for exoskeleton, in which all correlations are negative for both sexes. Pb and Co concentrations show a strong correlation in this tissue for male specimens, but no such association is seen for female specimens. In addition, the male cohort results display a strong correlation of Pb with Al and Ni as well as an

Table 3 Correlation rates between different metals within the same tissue type for hepatopancreas samples

	Length	CL	Weight	Al	^{52}Cr	^{53}Cr	Mn	Co	Ni	^{63}Cu	^{65}Cu	As	Mo	^{208}Pb	113Cd
Male															
Length	1														
CL	0.937**	1													
Weight	0.927**	0.917**	1												
Al	0.080	0.157	0.101	1											
^{52}Cr	−0.538**	−0.552**	−0.534**	−0.291	1										
^{53}Cr	−0.569**	−0.576**	−0.575**	−0.223	0.944**	1									
Mn	−0.202	−0.224	−0.247	0.110	0.199	0.206	1								
Co	−0.267	−0.285	−0.262	0.013	0.424**	0.472**	0.009	1							
Ni	−0.423**	−0.398*	−0.369*	−0.016	0.426**	0.435**	0.347*	0.500**	1						
^{63}Cu	−0.376*	−0.416*	−0.409*	0.061	0.601**	0.636**	0.191	0.299	0.209	1					
^{65}Cu	−0.496**	−0.514**	−0.492**	−0.156	0.700**	0.710**	0.189	0.388*	0.349*	0.896**	1				
As	−0.444**	−0.518**	−0.404*	−0.545**	0.651**	0.563**	0.253	0.195	0.551**	0.273	0.460**	1			
Mo	−0.402*	−0.472**	−0.375*	−0.242	0.776**	0.840**	0.186	0.567**	0.515**	0.561**	0.663**	0.589**	1		
^{208}Pb	−0.090	−0.030	−0.133	0.754**	0.001	0.030	0.244	−0.117	−0.072	0.404*	0.194	−0.375*	−0.259	1	
^{113}Cd	−0.248	−0.294	−0.213	−0.016	0.271	0.209	0.591**	0.258	0.355*	0.076	0.187	0.363*	0.319	−0.162	1
Female															
Length	1														
CL	0.957**	1													
Weight	0.903**	0.891**	1												
Al	0.356	0.392	0.143	1											
^{52}Cr	−0.279	−0.193	−0.181	−0.301	1										
^{53}Cr	−0.195	−0.106	−0.136	−0.384	0.914**	1									
Mn	0.215	0.236	0.082	0.349	−0.134	−0.190	1								
Co	0.034	0.020	0.053	−0.149	0.178	0.360	0.026	1							
Ni	−0.057	−0.060	−0.145	0.128	0.183	0.193	0.521*	0.628**	1						
^{63}Cu	0.118	0.243	0.124	0.124	0.464*	0.422*	0.196	−0.021	−0.083	1					
^{65}Cu	0.169	0.269	0.193	0.160	0.672**	0.645**	0.394	0.183	0.211	0.877**	1				
As	−0.086	−0.036	0.057	−0.203	0.612**	0.554**	0.251	0.300	0.579**	0.134	0.329	1			
Mo	−0.011	−0.039	0.026	−0.089	0.501*	0.535**	0.333	0.536**	0.722**	−0.003	0.304	0.847**	1		
^{208}Pb	0.213	0.324	0.041	0.523*	−0.151	−0.271	0.288	−0.229	−0.166	0.711**	0.518*	−0.355	−0.394	1	
^{113}Cd	0.131	0.221	0.267	−0.259	0.501*	0.497*	0.165	0.368	0.290	0.509*	0.575**	0.646**	0.396	−0.123	1

Significant results (* $P < 0.05$), particularly notable correlations (** $P < 0.01$)

CL carapace length excluding chelae

Table 4 Correlation rates between different metals within the same tissue type for abdominal muscle samples

	Length	CL	Weight	Al	^{52}Cr	^{53}Cr	Mn	Ni	^{63}Cu	^{65}Cu	As	Mo	111Cd	^{208}Pb
Male														
Length	1													
CL	0.937**	1												
Weight	0.927**	0.917**	1											
Al	−0.033	0.074	0.014	1										
^{52}Cr	−0.605**	−0.581**	−0.568**	−0.040	1									
^{53}Cr	−0.573**	−0.475**	−0.506**	0.220	0.552**	1								
Mn	0.211	0.252	0.138	0.745**	−0.136	−0.060	1							
Ni	0.259	0.338*	0.189	0.728**	−0.158	−0.087	0.923**	1						
^{63}Cu	−0.526**	−0.509**	−0.460**	0.244	0.529**	0.225	0.131	0.158	1					
^{65}Cu	−0.411*	−0.387*	−0.271	0.366*	0.429**	0.298	0.244	0.305	0.897**	1				
As	−0.206	−0.148	−0.197	0.551**	0.461**	0.139	0.560**	0.489**	0.495**	0.559**	1			
Mo	0.184	0.093	0.021	−0.109	−0.022	−0.260	0.217	0.175	−0.270	−0.172	0.152	1		
^{111}Cd	0.127	0.184	0.057	0.752**	−0.123	−0.017	0.964**	0.878**	0.190	0.250	0.586**	0.232	1	
^{208}Pb	0.013	0.074	−0.034	0.876**	−0.022	0.107	0.928**	0.894**	0.381*	0.445**	0.584**	0.045	0.917**	1
Female														
Length	1													
CL	0.957**	1												
Weight	0.903**	0.891**	1											
Al	0.330	0.334	0.223	1										
^{52}Cr	−0.148	−0.076	−0.093	0.160	1									
^{53}Cr	−0.244	−0.150	−0.368	0.197	0.551**	1								
Mn	0.522*	0.441*	0.463*	0.675**	0.168	0.182	1							
Ni	0.276	0.252	0.232	0.733**	0.317	0.424*	0.828**	1						
^{63}Cu	0.116	0.143	0.270	0.209	0.535**	−0.055	0.438*	0.361	1					
^{65}Cu	0.191	0.235	0.299	0.317	0.513*	0.022	0.534**	0.428*	0.965**	1				
As	0.442*	0.349	0.514*	0.422*	0.266	−0.032	0.780**	0.612**	0.553**	0.551**	1			
Mo	0.156	0.025	0.208	−0.086	−0.225	−0.283	0.014	−0.016	−0.287	−0.358	0.297	1		
^{111}Cd	0.567**	0.491*	0.528**	0.646**	0.170	0.155	0.945**	0.731**	0.390	0.510*	0.811**	0.082	1	
^{208}Pb	0.456*	0.409	0.421*	0.686**	0.280	0.226	0.971**	0.863**	0.541**	0.643**	0.733**	−0.121	0.902**	1

Significant results (* $P < 0.05$), particularly notable correlations (** $P < 0.01$)

CL carapace length excluding chelae

Table 5 Correlation rates of the same heavy metal across different tissue types

	Male					Female				
		Exo	Gills	Hepa	Muscle		Exo	Gills	Hepa	Muscle
Al	Exo	1				Exo	1			
	Gills	0.207	1			Gills	0.088	1		
	Hepa	0.188	0.013	1		Hepa	0.342	0.053	1	
	Muscle	0.106	−0.065	0.591**	1	Muscle	0.062	−0.174	0.367	1
As	Exo	1				Exo	1			
	Gills	0.623**	1			Gills	0.667**	1		
	Hepa	0.848**	0.547**	1		Hepa	0.602**	0.387	1	
	Muscle	−0.279	0.003	−0.133	1	Muscle	−0.240	0.033	−0.226	1
^{52}Cr	Exo	1				Exo	1			
	Gills	0.822**	1			Gills	0.732**	1		
	Hepa	0.782**	0.674**	1		Hepa	0.692**	0.708**	1	
	Muscle	0.499**	0.546**	0.562**	1	Muscle	−0.043	0.390	0.339	1
^{53}Cr	Exo	1				Exo	1			
	Gills	−0.081	1			Gills	−0.419*	1		
	Hepa	0.622**	0.057	1		Hepa	0.260	0.025	1	
	Muscle	0.313	0.172	0.448**	1	Muscle	0.036	0.207	0.013	1
^{63}Cu	Exo	1				Exo	1			
	Gills	0.489**	1			Gills	0.629**	1		
	Hepa	0.257	0.052	1		Hepa	0.106	0.122	1	
	Muscle	0.185	0.098	0.335*	1	Muscle	0.498*	0.112	0.185	1
^{65}Cu	Exo	1				Exo	1			
	Gills	0.681**	1			Gills	0.764**	1		
	Hepa	0.402**	0.332*	1		Hepa	0.174	0.289	1	
	Muscle	0.212	0.116	0.332	1	Muscle	0.202	0.042	0.261	1
Mn	Exo	1				Exo	1			
	Gills	0.108	1			Gills	0.444*	1		
	Hepa	0.047	0.471**	1		Hepa	0.158	0.182	1	
	Muscle	0.204	−0.378*	−0.091	1	Muscle	−0.038	0.058	0.237	1
Ni	Exo	1				Exo	1			
	Gills	0.807**	1			Gills	0.325	1		
	Hepa	0.451**	0.426**	1		Hepa	0.061	0.268	1	
	Muscle	−0.628**	−0.712**	−0.351*	1	Muscle	−0.426*	−0.532**	−0.0101	1

Metals not found in all tissues are not included. Significant results (* $P < 0.05$), ** particularly notable correlations (** $P < 0.01$)

Exo exoskeleton, *hepa* hepatopancreas

exceptionally strong correlation with Mn. Female specimens display similar results, with strong Pb–Ni and exceptionally strong Pb–Mn associations. Pb concentrations in gill tissues were lower than the detection limits, quite unlike most other tested elements, which were particularly concentrated in gill.

Another essential metal is Mn, and it is known that crayfish can accumulate high amounts of this metal in their tissues (Tunca et al. 2012). It is curious that Mn correlated with all elements tested except ^{53}Cr in gill tissue of male specimens, whereas for female specimens the only correlation present in the same tissue is with Ni. Exceptionally strong associations of Mn with Ni, Cd, and Pb were observed in male muscle tissue, with correlation rates greater than those between isotopes of the same metal. Similar associations are present between Mn and both Cd and Pb in female muscle tissue. Kurun et al. reported the Mn–Al correlation coefficient to be +0.79 in a study performed on *Astacus leptodactylus* (Terkos Lake) (Kurun et al. 2010). In the present study, correlation coefficients of Mn–Al in muscle tissue were found to be similar (+0.745 for male and +0.675 for female specimens), whereas no correlation was observed in hepatopancreas tissue for both sexes; a weak positive Mn–Al correlation was present in

Table 6 Correlation rates of different heavy metals across different tissue types

		Exo			Gills				Hepa			Muscle	
		Ni^{ab}	$^{63}Cu^{b}$	As^{ab}	$^{52}Cr^{b}$	Ni^{ab}	$^{63}Cu^{ab}$	As^{b}	Al^{b}	As^{a}	$^{52}Cr^{a}$	Mn^{a}	Ni^{a}
Exo	Ni^{ab}	1											
	$^{63}Cu^{a}$	0.617**	1										
	As^{ab}	0.783**	0.744**	1									
Gill	$^{52}Cr^{b}$	0.688**	0.695**	0.796**	1								
	Ni^{ab}	0.510**	0.639**	0.734**	0.688**	1							
	$^{63}Cu^{ab}$	0.571**	0.693**	0.767**	0.835**	0.817**	1						
	As^{b}	0.444**	0.528**	0.668**	0.732**	0.664**	0.689**	1					
Hepa	Al^{b}	−0.508**	−0.563**	−0.649**	−0.633**	−0.593**	−0.546**	−0.531**	1				
	As^{b}	0.516**	0.637**	0.794**	0.659**	0.760**	0.817**	0.445**	−0.495**	1			
	$^{52}Cr^{a}$	0.428**	0.487**	0.593**	0.670**	0.568**	0.588**	0.414**	−0.304*	0.726**	1		
Muscle	Mn^{a}	−0.524**	−0.453**	−0.664**	−0.520**	−0.575**	−0.591**	−0.346**	0.549**	−0.542**	−0.512**	1	
	Ni^{a}	−0.547**	−0.524**	−0.701**	−0.514**	−0.634**	−0.585**	−0.414**	0.508**	−0.577**	−0.541**	0.887**	1

Only the greatest correlation rates were presented as established by PCA. Significant results (* $P < 0.05$), particularly notable correlations (** $P < 0.01$)

Exo exoskeleton, *hepa* hepatopancreas

[a,b] The component group (group 1 or group 2, respectively) to which the variable in question belongs

Table 7 Elemental composition of sediment samples with focus on the heavy metals and metalloid tested

Si	Ca (%)	Fe (%)	Mg (%)	Al (%)	Mn (%)	Cr (%)	Cu (%)	Ni (%)	As (%)	Co (%)	Mo (%)	Ag (%)	Cd (%)	Hg (%)	Pb (%)
30.02	22.28	23.36	7.04	13.71	0.38	0.08	0.05	0.02	0.01	ND	ND	ND	ND	ND	ND
26.89	46.42	8.83	2.44	7.60	0.21	0.04	0.01	0.01	0.01	ND	ND	ND	ND	ND	ND
38.09	26.93	12.83	4.58	9.92	0.25	0.15	0.01	0.08	0.01	ND	ND	ND	ND	ND	ND

ND not determined (lower than detection limits)

Table 8 Bioaccumulation rates of the tested metals compared with their sediment concentrations

	Al	SD	^{52}Cr	SD	^{53}Cr	SD	Mn	SD
Exo	0.000897	0.000171	0.000845	0.000294	0.020185	0.006967	0.031534	0.006644
Gills	0.005216	0.001129	0.003795	0.001295	0.259445	0.088856	0.011258	0.002175
Hepa	0.000539	0.000122	0.000569	0.000197	0.044502	0.015485	0.054359	0.011186
Muscle	0.001379	0.000273	0.000367	0.000123	0.023217	0.007793	0.025146	0.005291
	Ni	SD	^{63}Cu	SD	^{65}Cu	SD	As	SD
Exo	0.002220	0.001110	1.145216	0.472308	0.657151	0.270096	0.201910	0.018138
Gills	0.005625	0.002856	1.111449	0.473752	1.003957	0.422086	0.253005	0.027014
Hepa	0.003080	0.001554	0.616139	0.258541	0.400761	0.167306	0.249050	0.033491
Muscle	0.000673	0.000339	0.705325	0.288882	0.508317	0.211019	0.021700	0.001896

Exo exoskeleton, *hepa* hepatopancreas

exoskeleton and gill for male specimens, but no such correlation was detected in those tissues for the female cohort.

Cr exists in valence states ranging from −2 to +6, with some oxidation states being essential (e.g., +3) and others toxic (e.g., +6) (Kwoczek et al. 2006; Bankar et al. 2009). ^{52}Cr and ^{53}Cr, the two isotopes of Cr inspected in this study, both display a moderately negative correlation with growth parameters in all tested tissue types in male specimens, whereas only a slight negative correlation in the exoskeleton samples was observed in female specimens for the same variables. It is interesting that there is a slight correlation between the two isotopes in gill and exoskeleton tissues of male specimens, whereas no such association can be observed in female specimens. Despite their low correlation ratios in "external" tissues, the two isotopes show a strong association in hepatopancreas and a moderate correlation in muscle tissue for both sexes. Both isotopes measured never displayed correlations with Al. ^{52}Cr accumulation was associated with ^{63}Cu, ^{65}Cu, and As in male specimens for all tissues; likewise, ^{52}Cr was correlated with ^{63}Cu and ^{65}Cu for all tissues of the female cohort. In muscle tissue, ^{53}Cr concentrations displayed no correlation with any other metal (except ^{52}Cr) for male specimens, whereas a single correlation was detected for female specimens. ^{52}Cr correlations in exoskeleton samples of male specimens were greatly similar to the correlation data associated with Co, As, Cu, and Ni, whereas ^{53}Cr was dissimilar to this group in that it lacked any correlation with Pb. Generally speaking, ^{52}Cr displayed correlations with a greater number of metals compared with ^{53}Cr. Male specimens' Cr–Ni correlations in hepatopancreas is similar to the results of Nakayama et al. (liver results) performed on Nile tilapia (*Oreochromis niloticus*), whereas Cr–Pb results are dissimilar (Nakayama et al. 2010).

Cu, an important metal for decapod crustaceans due to its incorporation into the oxygen-carrying protein hemocyanin, displayed completely different analysis results for the male and female cohorts, especially regarding growth parameters. Both Cu isotopes studied displayed a weak or moderate negative correlation with the morphological variables of male specimens, whereas no correlation was observed for Cu concentrations and any growth parameters of female tissues. ^{63}Cu and ^{65}Cu, the Cu isotopes measured in this study, had strong or exceptionally strong correlations with each other in all samples tested. Consequently, metals correlating with those two isotopes are mostly identical, with hepatopancreatic tissue displaying the lowest correlation between the two isotopes and therefore exhibiting the largest number of differences between the correlation characteristics of ^{63}Cu and ^{65}Cu. For both isotopes and both sexes, an association with Al was never observed, whereas ^{52}Cr displayed moderate to strong associations with Cu in all tissue types. A negative correlation was observed between Pb and ^{65}Cu in gill as well as

both ^{63}Cu and ^{65}Cu in muscle tissue, a result matching previous observations (Farkas et al. 2003). Generally, Cu isotopes in female specimens displayed fewer correlation trends with other metals compared with the male cohort. Correlations associated with Cu concentrations in exoskeleton samples of male specimens displayed large similarities with the correlations of Co, As, ^{52}Cr, and Ni.

It is known that Al is bioavailable and toxic to a variety of freshwater organisms (Alexopoulos et al. 2003; Ward et al. 2006). Compared with other elements tested, Al accumulation showed weaker and less numerous correlations, with the muscle tissue displaying the strongest results for this metal. Al concentrations in female specimens displayed fewer associations with other heavy metals compared with male specimens. The lack of correlation concerning this metal may be caused by its trivalency. Al + 3 is similar to Fe + 3 in structure and often competes with this metal for sites of sorption, effectively exploiting a "niche" unavailable and unrelated to other metals and metalloid tested. In addition, previous reports suggest that substantial amounts of accumulated Al were extracellular to the gill epithelium, which is associated with the mucus layer. This specific site of sorption may be the reason for the lack of correlation for Al (Alexopoulos et al. 2003).

It is curious that weight and size did not correlate with most heavy-metal concentrations. Some metals, such as Cr, Mn, and Mo, even exhibited negative weak correlations with growth parameters. This may be because the specimens displaying higher metal and metalloid bioaccumulation may experience growth retardation, resulting in small specimens with high amounts of heavy-metal and metalloid incorporation alongside larger, relatively heavy metal- and metalloid-free specimens. Inhibition of growth and regeneration capacity on heavy-metal exposure has previously been documented in crustaceans (Weis et al. 1992). Another explanation for this phenomenon is that it is an issue of selection, where specimens with particularly high heavy-metal intake rates perished before attaining large sizes and only individuals that could mostly avoid metal accumulation survived to reach large sizes. A previous study by Allinson et al. (2002) reported no correlation between length and As, Co, Cu, and Mn in gill of the Mozambique tilapia (*Oreochromis mossambicus*). Another study, performed by Cevik et al. (2008) on Mediterranean mussel (*Mytilus galloprovincialis*), reported an overall lower strength of correlation between heavy metals accumulated in the same tissue compared with our study in addition to a smaller number of associations in general. Smaller crayfish are known to ingest a greater proportion of animal matter, which may result in higher concentrations of the tested metals and metalloid in smaller individuals because those prey animals may also accumulate heavy metals from the environment (Gutierrez-Yurrita et al. 1998). In addition, heavy-metal accumulation is generally by food intake in lightly contaminated environments, and smaller individuals are consequently expected to contain comparatively greater amounts of heavy metals due to their more frequent feeding (Farkas et al. 2003). The lipid content of crayfish also changes by age, which may be another factor in explaining the negative correlation between heavy-metal concentrations and growth rate. Because adult specimens have greater lipid stores compared with juvenile specimens, the extra mass from this tissue might have offset our weight-based calculations. Further support for this observation is found in the general trend where female specimens do not display as many and strong negative correlations as do male specimens. This may be linked to the fact that the specimens were collected close to ovulation, a time where the female specimens have expanded most of their resources to facilitate egg development and therefore are lacking in lipid content compared with male specimens.

Another general trend observed in all samples was that the absorbed concentrations of metals with +2 as their dominant valance states tended to correlate with each other. For example, concentrations of Mn, Cu, and Ni, all sharing a dominant oxidation state of +2, were frequently correlated with each other in most of the tissue types tested. Likewise, concentrations in exoskeletal samples generally correlated with each other, with Co, As, ^{52}Cr, Ni, and Cu forming the primary correlation group. This may be caused by the availability of the crayfish carapace as a sorption site.

A substantial amount of fractionation was observed in the two primary isotopes of Cu (^{65}Cu, ^{65}Cu) and Cr (^{52}Cr, ^{53}Cr). Cr isotope fractionation has been recorded to occur during the reduction of hexavalent Cr to the trivalent form, a reaction readily performed in biological systems. Accordingly, the reduction of Cr(VI) to Cr(III) selects for the lighter isotope, resulting in lower ^{52}Cr/^{53}Cr ratios in the environment (and, conversely, higher ^{52}Cr/^{53}Cr ratios in tissues) as the reaction progresses (Sikora et al. 2008).

Correlation Trends of the Same Metal and Metalloid in Different Tissue Types

Because Cd, Pb, Mo, and Co concentrations were lower than detection limits in some tissue types, those metals were not analyzed in this section. Correlation trends of individual elements across the four tissue types tested are listed in Table 5.

Al concentrations display a moderate positive correlation between muscle and hepatopancreas samples of male specimens, whereas no such trend is present in female specimens. Abdominal muscle and the hepatopancreas are similar in that both tissue types have no connection to the

external environment, and although the former is generally not involved in Al accumulation, whereas the latter is a known site of biosorption (Madigosky et al. 1991), the fact that both accumulate this metal by absorbing it from a common source (hemolymph) might explain the correlation between the accumulation amounts.

As concentrations of gill samples in both sexes display a moderate positive correlation with both exoskeletal and hepatopancreatic As accumulation, whereas a strong correlation is present between the hepatopancreatic and exoskeletal As concentrations of male specimens. As thus appears to be able to penetrate into all tissues tested, and similar results were previously observed in fish tissues and blood (Maher et al. 1999). The fact that As can be converted to nontoxic organic forms may play a role in the correlations observed in all tissues (Fatrorini et al. 2006) because the relatively harmless organoarsenic compounds must be detoxified further and thus may occur anywhere in the body, leading to high correlations between all tissues. As amounts in gill tissues of female specimens also display a moderate correlation with the exoskeletal and hepatopancreatic accumulations of the same metal, although there is no correlation between hepatopancreas and exoskeleton results. This may be explained by the fact that the female crayfish collected were ovigerous and thus the formation of ova might have taken a toll on their other metabolic functions, resulting in lesser amounts of arsenic detoxification which in turn leads to the accumulation of this metal in hepatopancreas and a lack of correlation between this tissue and exoskeleton for As.

^{52}Cr concentrations correlate with each other in all tissues of male specimens, with the gill–exoskeleton association considered strong and the others moderate. No other tissue type correlates with muscle tissue in female specimens for this metal, whereas the concentrations in other three tissue types moderately correlate with each other. The lack of correlation for muscle tissue may be explained by the overall lack of Cr accumulation in this tissue (Bollinger et al. 1997). This is a result of the absence of binding molecules for storage in abdominal muscle (Guner 2007). Hepatopancreatic ^{53}Cr concentrations of male specimens moderately correlate with this metal's accumulation in both exoskeleton and muscles, whereas a negative correlation is observed between gill and exoskeleton samples in female specimens.

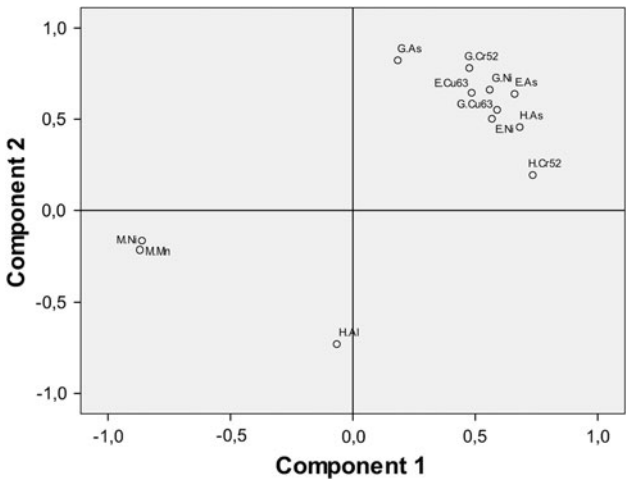

Fig. 1 PCA results of the variables with highest correlation rates with each other

Table 9 Rotated component matrix of PCA analysis

Component	1	2
Exo Ni	0.568	0.503
Exo ^{63}Cu		0.645
Exo As	0.660	0.639
Gills ^{52}Cr		0.781
Gills Ni	0.559	0.662
Gills ^{63}Cu	0.589	0.551
Gills As		0.823
Hepa Al		−0.729
Hepa ^{52}Cr	0.734	
Hepa As	0.681	
Muscle Mn	−0.862	
Muscle Ni	−0.870	

Only values with component loadings >0.5 are included

Exo exoskeleton, *hepa* hepatopancreas

Table 10 Accumulation equations for metals for which concentrations could be predicted with high confidence ($R^2 > 0.80$)

Regression models	R^2
$Exo_{65Cu} = 0.423(Exo_{63Cu}) + 0.071(Gills_{65Cu}) + 51.800$	0.885
$Exo_{As} = 0.002(Gills_{65Cu}) + 0.001(Exo_{63Cu}) - 0.001(Gills_{63Cu}) + 0.115(Hepa_{As}) + 1.148$	0.813
$Exo_{63Cu} = 1.802(Exo_{65Cu}) - 19.810$	0.867
$Gills_{65Cu} = 0.556(Gills_{63Cu}) + 130.642(Exo_{As}) + 24.817(Gills_{52Cr}) - 47.229(Hepa_{52Cr}) - 18.515(Hepa_{As}) - 181.686$	0.958
$Gills_{63Cu} = 1.413(Gills_{65Cu}) - 183.074(Exo_{As}) + 62.489(Hepa_{As}) - 21.135(Gills_{52Cr}) + 240.927$	0.966
$Gills_{52Cr} = 0.009(Gills_{65Cu}) + 0.708(Gills_{As}) + 1.392(Hepa_{52Cr}) - 0.004(Gill_{63Cu}) + 1.029$	0.835

R^2 = coefficient of determination

Table 11 Accumulation trends of various heavy metals and As in literature

Locale	Species	Heavy metals		Investigator
Guadiamar River, Spain	*P. clarkii*	As	H > EG > M	(Alcorlo et al. 2006)
		Cd	H > EG > M	
		Cu	H > M > EG	
		Pb	H > EG > M	
		Zn	H > EG > M	
Cache and Putah Creeks, United States	*P. leniusculus*	Hg	A > CT	(Hothem et al. 2007)
	P. clarkii	MeHg	A > CT	
		Trace elements (As, Cd, Pb, Se)	CT > A	
Louisiana, United States	*P. clarkii*	As	H > M > G > B	(Anderson et al. 1997)
		Cd	H > M > G > B	
		Cr	G > H > B > M	
		Cu	G > B > H > M	
		Pb	G > H > B > M	
Louisiana, United States	*P. clarkii*	Cu	C > A	(Richert and Sneddon, 2008)
		Fe	C > A	
		Zn	A > C	
Aras Dam, Iran	*A. leptodactylus*	Fe (M)	E > H > G > M = MC	(Naghshbandi et al. 2007)
		Fe (F)	E > G > H > MC > M	
		Cu (M)	H > G > E > M > MC	
		Cu (F)	G > H > E > M > MC	
		Zn (M)	H > G > MC > M > E	
		Zn (F)	H > G > MC > M > E	
		Mn (M)	E > H > G > MC > M	
		Mn (F)	E > G > H > MC > M	
		Pb (M)	E > G > H > MC > M	
		Pb(F)	G > E > H > MC > M	
		Cd (M)	E > H > G > MC > M	
		Cd (F)	G > E > H > MC > M	
Terkos Lake, Turkey	*A. leptodactylus*	Al (M)	G > M > H > Gn	(Kurun et al. 2010)
		Al (F)	G > Gn = H > M	
		Cd (M)	M > H = G > Gn	
		Cd (F)	H > H = G = Gn	
		Cu (M)	H > G > Gn > M	
		Cu (F)	H > G > Gn > M	
		Fe (M)	G > H > M > Gn	
		Fe (F)	G > M > H > Gn	
		Mn (M)	G > H > Gn > M	
		Mn (F)	G > H > Gn > M	
Hirfanlı Lake, Turkey	*A. leptodactylus*	Al	G > M > E > H	Present study
		As	H > G > E > M	
		^{52}Cr	G > H > E > M	
		^{53}Cr	G > H > M > E	
		^{63}Cu	E > G > H > M	
		^{65}Cu	G > H > E > M	
		Mn	H > E > M > G	
		Ni	G > H > M > H	

A abdomen, *B* hemolymph, *C* whole crayfish, *CT* whole crayfish, tail removed, *EG* exoskeleton and gills, *G* gills, *Gn* gonads, *H* hepatopancreas, *M* abdominal muscle, *MC* muscle of chelae

A moderate correlation is observed between gill and exoskeleton samples for ^{65}Cu in specimens of both sexes, whereas a weak association is present between muscle and hepatopancreas results of male specimens. For ^{65}Cu, all male tissues except abdominal muscle correlate with each other, whereas only a moderate association is observed between gill and exoskeleton of female specimens. ^{65}Cu and ^{65}Cu correlate between gill and exoskeleton of both sexes, with female tissues displaying higher correlation. The highest correlations are between gill and the exoskeleton, and both tissues are connected to the external environment. In addition, both tissues were previously reported as sites of high Cu biosorption (Soedarini et al. 2012).

Ni results correlate for each tissue type in male specimens, although the association is negative between muscle tissue and the other three. The gill–exoskeleton correlation is strong. A similar situation is observed for female specimens, with negative associations of Ni concentrations for abdominal muscle with gill and exoskeleton results. This result might indicate that Ni is mostly sorbed by gill or exoskeleton, which is supported by previous evidence that Ni mostly accumulates on the latter (Kouba et al. 2010).

Correlation Trends of Different Metals and Metalloid in Different Tissue Types

Table 6 displays the strongest correlations between the tested heavy metals and metalloid in different tissue types. PCA was used to combine the element-accumulation results. The strongest among those interactions, as observed by PCA, are given in Fig. 1 and Table 9. Two main component groups were observed by PCA. The results showed that the contribution ratio of the first principal component was 60.54 %, and the contribution ratio of the second principal component was 9.27 %. Those groups are treated together for the purpose of correlation analysis and are denoted as groups 1 and 2 in Table 6. Groups were constructed by only taking values with component loadings >0.5.

Male and female cohorts were not treated separately for the purpose of this analysis. Mn and Ni concentrations in abdominal muscle, as well as Al in exoskeleton, displayed a negative correlation with all other elements tested. Mn has previously been reported to negatively affect the absorption of many heavy-metal ions (Norwood et al. 2007), and a negative correlation might have resulted from this effect, whereas the relatively high concentration of Al in the surrounding sediment (Table 7) might have resulted in Al outcompeting other elements for binding sites, thus leading to negative correlations between Al and other metals. No other negative correlation was observed in the samples tested. Exoskeletal As concentrations display the highest correlation rates with metals of other tissue types; the strongest positive correlation observed was between ^{65}Cu in gill tissue and As in hepatopancreas samples, whereas the strongest negative correlation was between accumulated Ni in abdominal muscle and As in exoskeleton. Heavy metals in gill and exoskeleton samples displayed high correlation rates with each other, which might be caused by the constant contact both tissue types have with the outside environment. Environmental changes may therefore have similar impact on both tissues, resulting in the high correlation parameters observed.

Accumulation models are also listed in Table 10, using linear regression analysis for the elements, for which the models displayed $R^2 > 0.80$. Because both Cu isotopes displayed strong correlations with many of the elements tested, it is unsurprising that accumulation rates of other elements could be used to predict ^{65}Cu concentrations in gill and exoskeleton with high confidence, whereas ^{65}Cu accumulation could likewise be predicted in gill. Because As and Cu correlated with similar sets of elements, it is again unsurprising that As concentrations in exoskeleton related to Cu accumulation amounts. Crayfish tissue-accumulation trends of various metals previously reported in literature are briefly listed in Table 11 and generally are in line with our observations of hepatopancreas and gill as primary sorption sites.

Conclusion

The main aim of this study was to investigate of the accumulation effect of each metal and metalloid on the accumulation and the correlation trends of other metals, metalloid, and growth parameters. The strongest positive correlation observed for different metal and metalloid concentrations of different tissue types was between ^{65}Cu in gill and As in hepatopancreas. The strongest negative correlation was between exoskeletal As accumulation and muscle Ni amounts. Gill and the exoskeleton were the tissues with strongest correlations between each other for the greatest number of heavy metals. For correlation rates of individual heavy metals and metalloid across different tissues, As stands out for being found in high and correlating amounts in gills hepatopancreas and exoskeleton of male specimens. It is especially curious that although Pb and Cd isotopes had a very high correlation rates between themselves, this rate varied for isotopes of Cr and Cu, suggesting that isotope fractionation occurs during the accumulation of those two elements. Many divalent heavy metals displayed high correlations with each other. In many cases, weight and body lengths negatively correlated with metal and metalloid accumulations. Differences were also observed between correlation rate trends of male and female cohorts, and the accumulation modeling of some

metals and metalloid was determined for male and female specimens separately.

Bioindicator species is an important topic. The conclusions reached with this study have the potential of shedding light to studies to be performed on crayfish as a heavy-metal bioindicator in the future in both the laboratory and a natural ecosystem.

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References

Ahearn GA, Mandal PK, Mandal A (2004) Mechanisms of heavy-metal sequestration and detoxification in crustaceans: a review. J Comp Physiol B Biochem Syst Environ Physiol 174:439–452

Alcorlo P, Otero M, Crehuet M, Baltanás A, Montes C (2006) The use of the red swamp crayfish (*Procambarus clarkii*, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). Sci Total Environ 366(1):380–390

Alexopoulos E, McCrohan CR, Powell JJ, Jugdaohsingh R, White KN (2003) Bioavailability and toxicity of freshly neutralized aluminium to the freshwater crayfish *Pacifastacus leniusculus*. Arch Environ Contam Toxicol 45(4):509–514

Allinson G, Nishikawa M, De Silva SS, Laurenson LJ, De Silva K (2002) Observations on metal concentrations in tilapia (*Oreochromis mossambicus*) in reservoirs of south Sri Lanka. Ecotoxicol Environ Saf 51(3):197–202

Anderson M, Preslan J, Jolibois L, Bollinger J, George W (1997) Bioaccumulation of lead nitrate in red swamp crayfish (*Procambarus clarkii*). J Hazard Mater 54:15–29

Bankar AV, Kumar AR, Zinjarde SS (2009) Removal of chromium (VI) ions from aqueous solution by adsorption onto two marine isolates of *Yarrowia lipolytica*. J Hazard Mater 170:487–494

Bernhard M (1976) Manual of methods in aquatic environment research. Part 3: Sampling and analyses of biological material. Food and Agriculture Organization (FAO) Fish Technology Paper No. 158, UNEP, Rome, Italy

Bollinger JE, Bundy K, Anderson MB, Millet L, Preslan JE, Jolibois L et al (1997) Bioaccumulation of chromium in red swamp crayfish (*Procambarus clarkii*). J Hazard Mater 54:1–13

Cevik U, Damla N, Kobya AI, Bulut VN, Duran C, Dalgic G et al (2008) Assessment of metal element concentrations in mussel (*M. galloprovincialis*) in Eastern Black Sea, Turkey. J Hazard Mater 160(2–3):396–401

Farkas A, Salánki J, Specziár A (2003) Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. Water Res 37:959–964

Fatrorini D, Notti A, Regoli F (2006) Characterization of arsenic content in marine organisms from temperate, tropical, and polar environments. Chem Ecol 22(5):405–414

Gadzala-Kopciuch R, Berecka B, Bartoszewicz J, Buszewski B (2004) Some considerations about bioindicators in environmental monitoring. Pol J Environ Stud 13(5):453–462

Guner U (2007) Freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823) accumulates and depurates copper. Environ Monit Assess 133(1–3):365–369

Gutierrez-Yurrita P, Sancho G, Bravo M, Baltanas A, Montes C (1998) Diet of the red swamp crayfish *Procambarus clarkii* in natural ecosystems of the Donana National Park temporary fresh-water marsh (Spain). J Crustacean Biol 18(1):120–127

Hothem RL, Bergen DR, Bauer ML, Crayon JJ, Meckstroth AM (2007) Mercury and trace elements in crayfish from Northern California. Bull Environ Contam Toxicol 79:628–632

Khan S, Nugegoda D (2003) Australian freshwater crayfish *Cherax destructor* accumulates and depurates nickel. Bull Environ Contam Toxicol 70(2):308–314

Kouba A, Buric M, Kozak P (2010) Bioaccumulation and effects of heavy metals in crayfish: a review. Water Air Soil Poll 211 (1–4):5–16

Kurun A, Balkıs N, Erkan M, Balkıs H, Aksu A, Erşan MS (2010) Total metal levels in crayfish *Astacus leptodactylus* (Eschscholtz, 1823), and surface sediments in Lake Terkos, Turkey. Environ Monit Assess 169(1–4):385–395

Kwoczek M, Szefer P, Hac E, Grembecka M (2006) Essential and toxic elements in seafood available in Poland from different geographical regions. J Agric Food Chem 54(8):3015–3024

Lacerda LD, Santos JA, Lopes DV (2009) Fate of copper in intensive shrimp farms: bioaccumulation and deposition in pond sediments. Braz J Biol 69(3):851–858

Lopez FJS, Garcia MDG, Vidal JLM, Aguilera PA, Garrido Frenich A (2004) Assessment of metal contamination in Doñana National Park (Spain) using crayfish (*Procamburus clarkii*). Environ Monit Assess 93:17–29

Madigosky SR, Alvarez-Hernandez X, Glass J (1991) Lead, cadmium, and aluminum accumulation in the red swamp crayfish *Procambarus clarkii* G. collected from roadside drainage ditches in Louisiana. Arch Environ Contam Tox 20(2):253–258

Maher W, Goessler W, Kirby J, Raber G (1999) Arsenic concentrations and speciation in the tissues and blood of sea mullet (*Mugil cephalus*) from Lake Macquarie NSW, Australia. Mar Chem 68(1–2):169–182

Naghshbandi N, Zare S, Heidari R, Razzaghzadeh S (2007) Concentration of heavy metal in different tissues of *Astacus leptodactylus* from Aras Dam of Iran. Pakistan J Biol Sci 10(21): 3956–3959

Nakayama SMM, Ikenaka Y, Muzandu K, Choongo K, Oroszlany B, Teraoka H et al (2010) Heavy metal accumulation in lake sediments, fish (*Oreochromis niloticus* and *Serranochromis thumbergi*), and crayfish (*Cherax quadricarinatus*) in Lake Itezhi-Tezhi and Lake Kariba, Zambia. Arch Environ Contam Toxicol 59(2):291–300

Norwood WP, Borgmann U, Dixon DG (2007) Interactive effects of metals in mixtures on bioaccumulation in the amphipod *Hyalella azteca*. Aquat Toxicol 84(2):255–267

Richert JC, Sneddon J (2008) Determination of heavy metals in crawfish (*Procambrus clarkii*) by inductively coupled plasma–optical emission spectrometry: a study over the season in southwest Louisiana. Anal Lett 41(17):3198–3209

Sikora E, Johnson T, Bullen T (2008) Microbial mass-dependent fractionation of chromium isotopes. Geochim Cosmochim Acta 72(15):3631–3641

Soedarini B, Klaver L, Roessink I, Widianarko B, Van Straalen NM, Van Gestel CA (2012) Copper kinetics and internal distribution in the marbled crayfish (*Procambarus* sp.). Chemosphere 87(4):333–338

Tunca E, Atasagun S, Saygi Y (2012) Yeniçağa Gölü'nde (Bolu-TÜRKİYE) Su, Sediment ve Kerevitteki (*Astacus leptodactylus*) Bazı Ağır Metallerin Birikimi Üzerine Bir Ön Çalışma. Ekoloji 21:68–76

Varmuza K, Filzmoser P (2009) Introduction to multivariate statistical analysis in chemometrics. CRC Press, Boca Raton, Florida

Ward R, McCrohan C, White K (2006) Influence of aqueous aluminium on the immune system of the freshwater crayfish *Pacifasticus leniusculus*. Aquat Toxicol 77(2):222–228

Weis J, Cristini A, Rao K (1992) Effects of pollutants on molting and regeneration in crustacea. Am Zool 32(3):495–500

Wigginton AJ, Birge WJ (2007) Toxicity of cadmium to six species in two genera of crayfish and the effect of cadmium on molting success. Environ Toxicol Chem 26(3):548–554

Yilmaz A, Yilmaz L (2007) Influences of sex and seasons on levels of heavy metals in tissues of green tiger shrimp (*Penaeus semisulcatus* de Hann, 1844). Food Chem 101(4):1664–1669